UNIVERSITI PUTRA MALAYSIA

CYTOKINE PRODUCTION BY A HUMAN ENDOTHELIAL CELLLINE IN RESPONSE TO CANDIDA ALBICANS

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CYTOKINE PRODUCTION BY A HUMAN ENDOTHELIAL CELL LINE IN RESPONSE TO *CANDIDA ALBICANS*

By

LIM PEI CHING

Thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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DEDICATION

To my parents, who put up with me, and

Jin Hoong, who encourage and accompany me always.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

CYTOKINE PRODUCTION BY A HUMAN ENDOTHELIAL CELL LINE IN RESPONSE TO *CANDIDA ALBICANS*

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October 2005

Chairman: Professor Seow Heng Fong, PhD

Faculty: Medicine and Health Sciences

Candida albicans is the most common aetiological agent that causes haematogenously disseminated candidiasis. Under conditions that compromise the host immune system, *C. albicans* disseminates from mucosal sites and results in a progressive disease associated with high rates of mortality. Cytokines are important immunomodulators in coordinating the host defense against *C. albicans* infection. Human endothelial cells are known to produce various types of cytokines in response to pathogen invasion. The present study was undertaken to identify the cytokines that are involved in the host defense against *C. albicans*, as well as, to determine the importance of direct cell-to-cell contact in triggering expression of cytokines. In addition, the involvement of Toll-like receptor (TLR)2, TLR4 and nuclear factor-κB (NF-κB) in the host defense against *C. albicans* were also examined. Expression of cytokines by endothelial cells in response to *C. albicans* was investigated by using an *in vitro* model of human umbilical vein endothelial cell line (HUVEC) co-cultured with *Candida* spp. Both conventional and real time PCR showed that among the cytokines studied, only granulocyte-macrophage colony-stimulating factor (GM-CSF) was found to be differentially expressed in

HUVEC upon stimulation with *C. albicans*. Elevated levels of GM-CSF were found in the co-culture of HUVEC with *C. albicans* but not in the other non-albicans *Candida* spp. Three additional *C. albicans* strains co-cultured with HUVEC also showed a similar pattern of increased GM-CSF expression, although at different levels from strain to strain. This provided evidence that the induction of GM-CSF was not confined to only a particular clinical strain of *C. albicans*. On the other hand, *C. dubliniensis*, which possessed a similar phenotype as *C. albicans* failed to stimulate a similar pattern of GM-CSF expression in HUVEC. The induction of GM-CSF was then found to be contact-dependent via the use of cell culture insert to physically separate *C. albicans* from adhering to the HUVEC monolayer. Pretreatment with anti-TLR2 and anti-TLR4 antibodies showed that TLR4 but not TLR2 was involved in the induction of GM-CSF expression by HUVEC. In addition, pretreatment with SN50 inhibitor also demonstrated that NF-κB may be involved in stimulating expression of GM-CSF transcript. In conclusion, we have discovered that HUVEC is involved in the innate immune response to *C. albicans* by producing GM-CSF cytokine through the activation of TLR4 and also NF-κB transcription factor in a contact-dependent manner.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGZAHIRAN SITOKIN DI DALAM JUJUKAN SEL MANUSIA ENDOTELIUM YANG DIARUH OLEH *CANDIDA ALBICANS*

Oleh

LIM PEI CHING

Oktober 2005

Pengerusi: Profesor Seow Heng Fong, PhD

Fakulti: Perubatan dan Sains Kesihatan

Candida albicans adalah punca penyakit kandidiasis yang menyebar melalui darah. Apabila sistem pertahanan badan menjadi lemah, *C. albicans* boleh menyebar melalui mukosa ke dalam organ dalaman dan menyebabkan penyakit kandidiasis menjadi semakin serius dan membawa kepada kadar kematian yang tinggi. Sitokin penting dalam mengkoordinasikan sistem pertahanan untuk melawan jangkitan *C. albicans.* Jujukan sel manusia endotelium diketahui boleh menghasilkan pelbagai sitokin untuk melawan serangan patogen. Tujuan penyelidikan ini adalah untuk mengenalpasti sitokin yang terlibat dalam sistem pertahanan yang melawan serangan *C. albicans* dan untuk mengkaji kepentingan sentuhan dalam jujukan sel manusia endotelium (HUVEC) dan *C. albicans* dalam pengzahiran sitokin. Tambahan pula, penglibatan 'Toll-like receptor' (TLR)2 dan TLR4 serta Factor nuklear-κB (NF-κB) dalam sistem pertahanan terhadap *C. albicans* juga dikaji dalam penyelidikan ini. Pengzahiran sitokin dalam HUVEC yang diaruh oleh *C. albicans* dilakukan melalui penggunaan sebuah model kultur luaran antara HUVEC dengan *C. albicans*. Kedua-dua teknik tradisional dan "real time" RT-PCR telah menunjukkan di antara sitokin-sitokin yang disiasat, hanya granulocyte-

macrophage colony-stimulating factor (GM-CSF) sahaja yang diaruhkan secara berbeza oleh *C. albicans*. Peningkatan pengzahiran GM-CSF hanya berlaku di dalam kultur model HUVEC dengan *C. albicans* dan bukannya dengan spesies *Candida* yang lain. Tiga tambahan *C. albicans* yang lain juga dikulturkan dengan HUVEC dan menunjukkan cara pengzahiran GM-CSF yang sama dan mengesahkan pengzahiran GM-CSF adalah bukan disebabkan oleh *C. albicans* yang tertentu sahaja. Di samping itu, *C. dubliniensis* yang mempunyai sifat yang sama dengan *C. albicans* telah gagal mengaruh pengzahiran GM-CSF di dalam HUVEC. Pengaruhan GM-CSF didapati bergantung kepada sentuhan antara HUVEC dengan *C. albicans* apabila sebuah pengisi kultur sel digunakan untuk memisah secara fizikal *C. albicans* daripada melekat pada HUVEC. Penggunaan anti-TLR2 antibodi dan anti-TLR4 antibodi menunjukkan bahawa TLR4 dan bukan TLR2 yang terlibat dalam pengzahiran GM-CSF yang diaruhkan oleh *C. albicans*. Sementara itu, SN50 menunjukkan pengzahiran GM-CSF juga bergantung kepada faktor transkripsi NF-κB yang bertanggungjawab dalam memulakan transkripsi GM-CSF sitokin. Kesimpulannya, HUVEC adalah terlibat dalam sistem pertahanan badan yang tidak terancang apabila dirangsangkan oleh *C. albicans* dengan pengzahiran GM-CSF melalui pengaktifan TLR4 serta factor transkripsi NF-κB yang bergantung kepada kewujudannya sentuhan antara HUVEC dengan *C. albicans*.

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.I certify that an Examination Committee met on 28th October 2005 to conduct the final examination of Lim Pei Ching on her Master of Science thesis entitled "Cytokine Production by a Human Endothelial Cell Line in Response to *Candida albicans*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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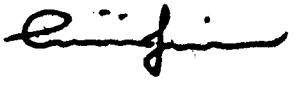

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Date: 07 FEB 2006

DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

LIM PEI CHING

Date: 17 Jan 2006

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~	approximately
α	alpha
β	beta
γ	gamma
Δ	delta
κ	kappa
cm	centimeter
g	gram
µg	microgram
pg	picogram
µl	microliter
µm	micrometer
mg	milligram
mM	millimolar
ml	millimiter
nm	nanometer
°C	degree of Celsius
%	percentage
V	volt
bp	base-pair
kb	kilobase-pair

L	liter
ALS	Agglutinin-Like-Sequence
AP	Alkaline Phosphatase
Arg	Arginine
Asp	Aspartic acid
ATCC	American Type Culture Collection
β2M	beta-2-microglobulin
cDNA	complementary Deoxyribonucleic acid
CGM	completed growth medium
CO_2	carbon dioxide
CSF	colony-stimulating factor
C_T	threshold cycle
DEPC	diethyl pyrocarbonate
dNTPs	dideoxynucleotide triphosphates
ECGS	Endothelial Cells Growth Supplement
EDTA	ethylenediamine tetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
FBS	Foetal Bovine Serum
Gly	Glycine
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
HBSS	Hank's Balance Salts Solution
HUVEC	Human Umbilical Vein Endothelial Cells
HWP-1	Hyphal wall protein-1